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Enhancing *Clitoria ternatea* Propagation Through Indirect Somatic Embryogenesis: Optimal Plant Growth Regulators Type and Concentration for Efficient in Vitro Plant Production

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Abstract:- The propagation of *Clitoria ternatea* poses several challenges, including low germination rates, vulnerability of young seedlings in natural environment, and a slow flowering rate. This study aims to identify the best Plant Growth Regulators and their optimal concentration to stimulate indirect somatic embryogenesis from *C. Ternatea* leaves explants. The samples were cultured on Murashige and Skoog (MS) medium supplemented with various concentration of thidiazuron (TDZ; 1, 2, 3, 4, and 5 mgL^{-1}) and a combination of 2,4-dichloro phenoxy acetic acid (2,4-D; 1, 2, and 3 mgL^{-1}) and 0.1 mgL^{-1} kinetin. The findings revealed that all treatments resulted in the formation of both embryogenic and non embryogenic calluses. The addition of TDZ to the MS medium led to a greater percentage of embryogenic callus formation compared to using the combination of kinetin and 2,4-D. The highest percentage of 84.37% was achieved when samples were cultured on MS medium with 3 mgL^{-1} TDZ. The most effective induction period of 21 days was observed with the medium supplemented with 3 mgL^{-1} TDZ, followed by 4 and 5 mgL^{-1} TDZ, which took 23 days. The research findings suggest that a concentration of 3 mgL^{-1} TDZ is the optimal PGR type and dosage for achieving rapid and efficient in vitro plant production compared to various concentrations of 2,4-D in combination with kinetin.

Keywords:- *Butterfly Pea, Dichlorophenoxyacetic Acid, Embryogenesis, Thidiazuron.*

I. INTRODUCTION

Clitoria ternatea, referred to as butterfly pea or *bunga telang* in Indonesia, is a perennial ornamental herbaceous plant belonging to the Fabaceae family. It is characterized by its striking white and blue flowers [1,2]. Lately, it has gained significant attention due to its medical uses and agricultural applications. These applications span from being utilized as a cover crop or green manure to its roles in food coloring, cosmetics, and environmentally friendly insecticides [3,4,5]. In traditional medicine, various components of *C. ternatea* have been recognized for their antiasthmatic, pain-relieving, anti-inflammatory, antipyretic, and antioxidant properties [6,7,8]. The extract from *C. ternatea* flowers was found to effectively reduce the activity of 2,2-diphenylpicrylhydrazyl (DPPH) radicals [9]. In a different research study, it was found that oral administration of ethanolic extracts from *C. ternatea* flowers significantly reduced rat paw swelling induced by carrageenan in rats [10].

The continuous demand for medicinal plants, along with habitat destruction, threatens various plant species critical to ecosystems and human welfare. *C. ternatea*, in particular, faces increasing pressure due to its emerging commercial value and demand for its phytochemical compounds [11,12]. The wild population of *C. ternatea* is declining due to destructive harvesting, ongoing deforestation, and a lack of efforts to replenish its numbers [13]. Traditional cultivation of *C. ternatea* using seeds faces challenges such as low germination rates and the mortality of young seedlings in natural settings [14]. Therefore, there is a need for widespread propagation of *C. ternatea*, along with efforts to conserve this plant as a crucial genetic resource. This initiative is essential to support both pharmacological research and genetic improvement programs.

In vitro culture provides a biotechnological solution for the propagation, conservation, and study of plant responses and natural secondary metabolites, serving as an alternative method for both proliferation and conservation of the plant [15,16]. Cultivating callus in vitro offers a sustainable method for isolating phytochemical compounds from *C. ternatea* without causing excessive depletion [12]. Several studies have documented the micropropagation of diverse medicinal plants [17,18,19].

The capacity of plant cells to undergo reprogramming via somatic embryogenesis has been acknowledged as a potent method. This process enables vegetative propagation with higher genetic uniformity compared to seed propagation and serves as a fundamental protocol for propagating species that are rare, endangered, and challenging to cultivate [13,20]. A successful somatic embryogenesis convention requires suitable physical and chemical variables including

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temperature, humidity, light administration, media formulations and particular plant growth regulators (PGRs) [21,22,23]. Several reports about utilizing PGRs for Somatic embryogenesis protocols in *C. ternatea* using nodal segments, immature embryo and seedlings [24,25,26].

Principally, the addition of PGRs like cytokinins and auxins into in vitro media is essential to stimulate somatic embryo formation, either indirectly or directly by intervening during the callus phase. One of the most commonly utilized inducers of somatic embryogenesis is 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic PGR with auxin activity [26]. Its effects on plants are long-lasting due to its high stability within plant cells [27]. Numerous studies have demonstrated that 2,4-D can regulate indoleacetic acid (IAA) metabolism, control DNA methylation, and induce specific proteins in tissue cultures [28,29]. Moreover, it is thought that 2,4-D influences the development of plants by eliciting stress responses, especially in the process of reprogramming somatic cells towards the embryogenic pathway [30,31]. In a separate study, it was observed that approximately 75% of cotyledon explants from *C. ternatea* formed embryogenic callus when planted on a medium containing 2 mgL^{-1} 2,4-D [13].

Thidiazuron (TDZ) is a powerful artificial growth controller that demonstrates effects in plants similar to cytokinins, resulting in a diverse array of in vitro uses, such as the multiplication of unexpected shoots and the stimulation of somatic embryogenesis [32]. The potent cytokinin impact of TDZ is linked to its exceptional capability to enhance natural cytokinin levels within plants and its resistance to degradation by cytokinin oxidase [33]. Besides TDZ, kinetin is also another substitute for cytokinin requirement that utilized during somatic embryogenesis induction. Although TDZ is recognized for its ability to trigger somatic embryogenesis either independently or in conjunction with other plant growth regulators (PGRs), kinetin is typically combined with auxins in the growth medium.

Certain research has shown that the induction of somatic embryo development in the *Phalaenopsis amabilis* orchid can be effectively achieved through the sole use of TDZ [22]. Conversely, the combined application of kinetin and 2,4-D triggers indirect somatic embryogenesis in the medicinal plant Bergenia ciliata [11]. In this study, different levels of TDZ and 2,4-D in combination with kinetin were examined to determine the optimal type and concentration of PGRs for inducing somatic embryogenesis in *C. ternatea*. The resulting protocol will find practical applications in *C. ternatea* breeding and genetic modification.

II. MATERIALS AND METHODS

A. Plant Specimen and Preparation of Samples

Our research was carried out at the Biotechnology Laboratory, Universitas Atma Jaya Yogyakarta. The study involved the use of leaves obtained from *C. ternatea* trees harvested in the Sleman, Yogyakarta region (Indonesia). Newly harvested leaves were disinfected with 70% ethanol for half a minute and afterward washed with sterile distilled water. Following this, they underwent a pretreatment with fungicide and bactericide for 5 minutes, and 25% sodium hypochloride (NaClO) for 3 minutes. The leaves were rinsed with sterile distilled water after each pretreatment. The explants were transversely cut and positioned on a solid Murashige and Skoog (MS) medium.

B. Experimental Design

A total of 256 samples of plant material were randomly divided into 8 different treatment groups. Each group comprised 32 specimens that were cultivated in 8 Petri dishes (60 x 10 mm), with each Petri dish containing 4 samples. The medium was supplied with increasing concentrations of TDZ (1, 2, 3, 4, and 5 mgL^{-1}) or 2,4-D (1, 2, and 3 mgL^{-1}) combined with 0.1 mgL^{-1} kinetin. The cultures were kept in the absence of light at a temperature of 26 ±1 degrees Celsius. Subcultures were conducted every four weeks. After 8 weeks, cultures were transferred to a photoperiod conditions of 8 hours light and 16 hours dark. The effect of the PGRs' composition on the induction time, percentage, development pattern, texture, and color of embryogenic callus formation was monitored weekly for five weeks using a dissecting microscope. Photographs were taken using Optilab microscope camera (Miconos).

C. Data Analysis

The data were statistically analyzed with an analysis of variance (ANOVA). The comparison of means was conducted using the Duncan multiple range test at a confidence level of $P < 0.05$.

III. RESULTS AND DISCUSSION

A. Somatic Embryogenesis Induced by Various Concentrations of PGRs

Distinct responses in somatic embryo induction were observed in leaf explants of *C. ternatea* when exposed to eight different media for somatic embryo induction. Successful induction of somatic embryogenesis was achieved in the medicinal plant *C. ternatea* through this investigation. All tested concentrations of TDZ [Table 1] and the combination of kinetin and 2,4-D [Table 2] demonstrated the capability to induce embryogenic callus from leaf explants. This observation is consistent with prior research that underscores the application of exogenous cytokinin and auxin for stimulating the development of callus in diverse plant species [34]. The fate of tissue morphogenesis is predominantly influenced by the auxin-to-cytokinin ratio, with a higher ratio of auxin/cytokinin promoting root regeneration, while the opposite ratio promotes shoot regeneration. In concurrence with this finding, another study also documented the successful propagation of a medicinal plant at risk, *Kelussia odorotissima*, using somatic embryogenesis. Their study revealed that a combination of 0.25 mg/L kinetin and 1 mg/L 2,4-D resulted in a high percentage of embryogenic callus formation from leaf explants [35].

Although both TDZ and the combination of kinetin and 2,4-D can initiate somatic embryogenesis, in this study, the percentage of somatic embryos was significantly higher when using TDZ compared to the medium added with kinetin and

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2,4-D. The findings revealed that the greatest proportion of somatic embryo formation (84.375%) occurred in leaf explants cultured on MS medium with an addition of 3 mgL^{-1} TDZ [Table 1]. Meanwhile, the promptest formation of somatic embryos (21 days) was attained when samples were placed on MS medium with the addition of TDZ at a concentration of 5 mgL^{-1}. Although treatments involving the composite of kinetin and 2,4-D also triggered somatic embryogenesis, the percentage was relatively low [Table 2]. It was noted that using 1 mgL^{-1} kinetin along with 2 mgL^{-1} 2,4-D resulted in the generation of only 43.75% of somatic embryos from the specimens. Meanwhile, the most rapid development of somatic embryos, observed in the combination of 0.1 mgL^{-1} kinetin + 3 mgL^{-1} 2,4-D, occurred 32 days after the start of the culture.

Table 1 Effects of Various Concentrations of TDZ on the Formation of Somatic Embryos from Leaf Explants of *C. Ternatea*

Concentrations of TDZ (mgL^{-1})	**Somatic embryogenesis induction time (days)**	**Percentage of somatic embryos formation (%)**	**Percentage of non-embryogenic callus formation (%)**
1	25	56.25	25.00
2	25	78.12	6.25
3	21	84.37	3.12
4	23	46.87	3.12
5	23	56.25	3.12

Table 2 Effects of Combination of 2,4-D and Kinetin on the Formation of Somatic Embryos from Leaf Explants of *C. Ternatea*

Growth regulators (mgL^{-1})		**Somatic embryogenesis induction time (days)**	**Percentage of somatic embryos formation (%)**	**Percentage of non-embryogenic callus formation (%)**
2,4 D	**Kinetin**			
1	0.1	34	21.88	43.75
2	0.1	32	43.75	18.75
3	0.1	34	37.50	37.50

Earlier research supporting this discovery noted that both indirect and direct somatic embryogenesis were successfully induced from nodal explants of *Anoectochilus elatus* orchid using TDZ alone. Other plant growth regulators (BA, 2iP, NAA, 2,4-D) resulted in a fewer somatic embryos with a reduced frequency of embryo initiation [36]. Furthermore, it was also reported that the addition of 0.12 mgL^{-1} TDZ increased the level of somatic embryo production up to 22% in the herbal plant *Eurycoma longifolia* [37]. Our data also support findings indicating that somatic embryogenesis in leaf explants of the Oncidium 'Gower Ramsey' orchid was hindered by external auxins, but enhanced by cytokinins [38].

It has been known that TDZ, a phenylurea-type cytokinin, is a potent agent in plant tissue cultivation. TDZ influences the embryogenic response of explants by participating in the regulation of the cell cycle and the metabolism of endogenous phytohormones, either as a consequence of induced stress or directly [39,40]. TDZ enhances the availability of purines for cellular growth and facilitates the conversion of adenine to adenosine [41]. These processes are crucial during protein synthesis and cell division, both of which are rapid processes occurring in somatic embryogenesis. TDZ has been identified as a pivotal factor for inducing somatic embryogenesis and regenerating buds/shoots [40,42].

According to the percentage data for somatic embryo formation after 5 weeks of cultivation, it was observed that an increase in TDZ concentration led to a decrease in the percentage of non-embryogenic callus formation. Specifically, the non-embryogenic callus percentage dropped to 3.12% when the culture medium was supplemented with TDZ concentrations ranging from 3 mgL^{-1} to 5 mgL^{-1} [Table 1]. In contrast, when considering the combination of kinetin and 2,4-D [Table 2], a higher percentage of non-embryogenic callus was formed in MS medium supplemented with 0.1 mgL^{-1} kinetin and 1 mgL^{-1} 2,4-D (43.75%), followed by 0.1 mgL^{-1} kinetin + 3 mgL^{-1} 2,4-D (37.50%).

B. Callus Morphhogenesis

A morphogenetic activity, marked by the growth of both non-embryogenic and embryogenic callus, was observed in different treatment media. In TDZ-containing medium, explants began to swell after two weeks of induction [Figure 1]. Similar outcomes were achieved in cultures sustained on MS medium enriched with a combination of kinetin and 2,4-D, as depicted in [Figure 2]. Embryogenic callus was then observed with friable structure and light green in color appeared from the surface near the wounding site of leaf explants [Figure 3]. The non-embryogenic callus exhibited colors ranging from translucent whitish to green and blackish brown, with textures varying between friable and compact.

Explants planted on MS medium added with 0.1 mgL^{-1} kinetin and 1 mgL^{-1} 2,4-D produced combination of both friable green and brown calli. While, explants planted on 0.1 mgL^{-1} kinetin in combination with 2 mgL^{-1} 2,4-D produced in friable, white, transparent, and wet looking callus [Figure 3]. In contrast, a combination of 0.1 mgL^{-1} kinetin along with 3 mgL^{-1} 2,4-D induced the formation of compact, white, and blackish brown callus. Non-embryogenic callus can not developed into somatic embyos and eventually became necrotic. Whereas, embryogenic callus further developed into somatic embryos and regenerated into plants.

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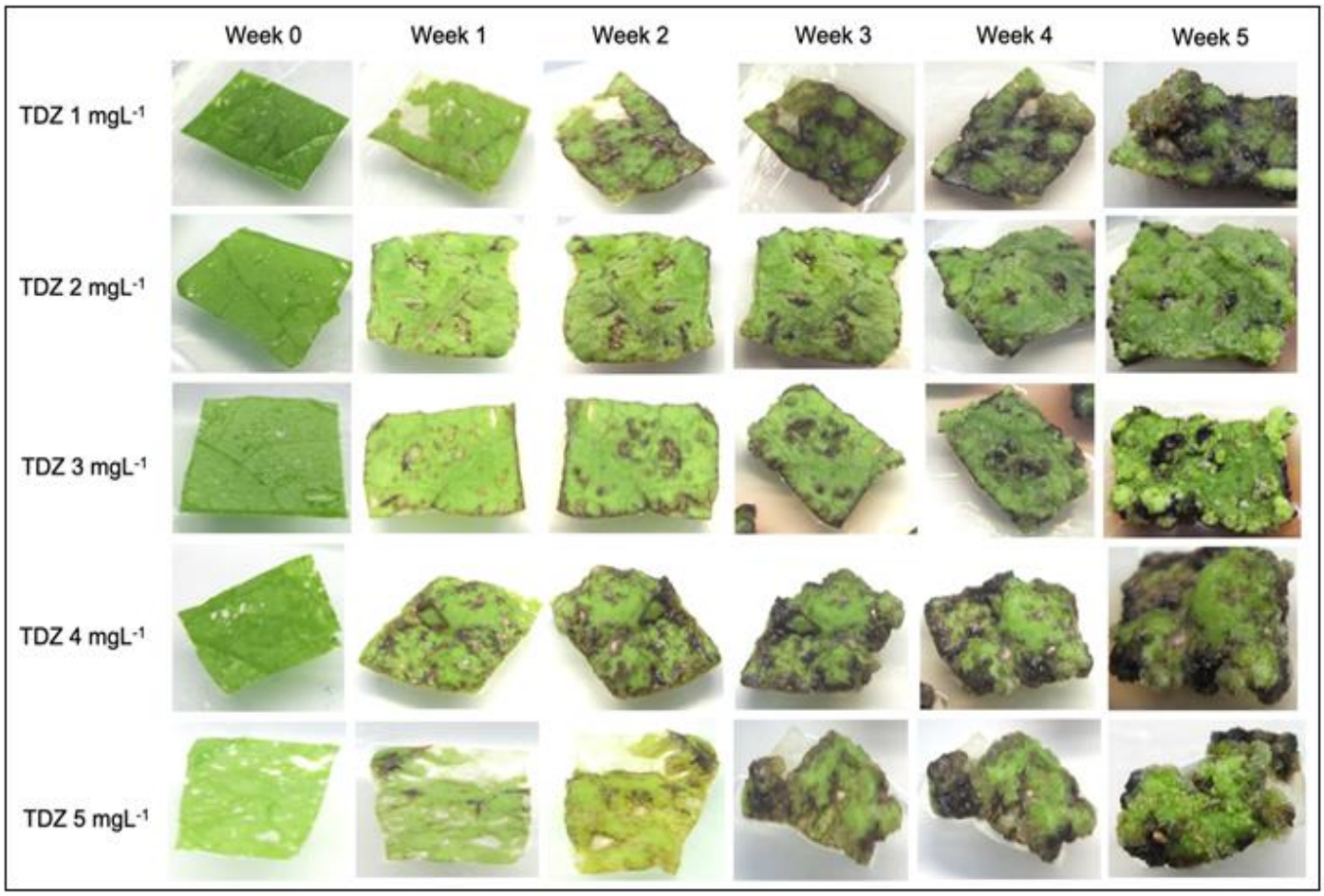

Fig 1 Effect of Different TDZ Concentrations on the Indirect Somatic Embryogenesis from Leaf Explants of *C. ternatea*

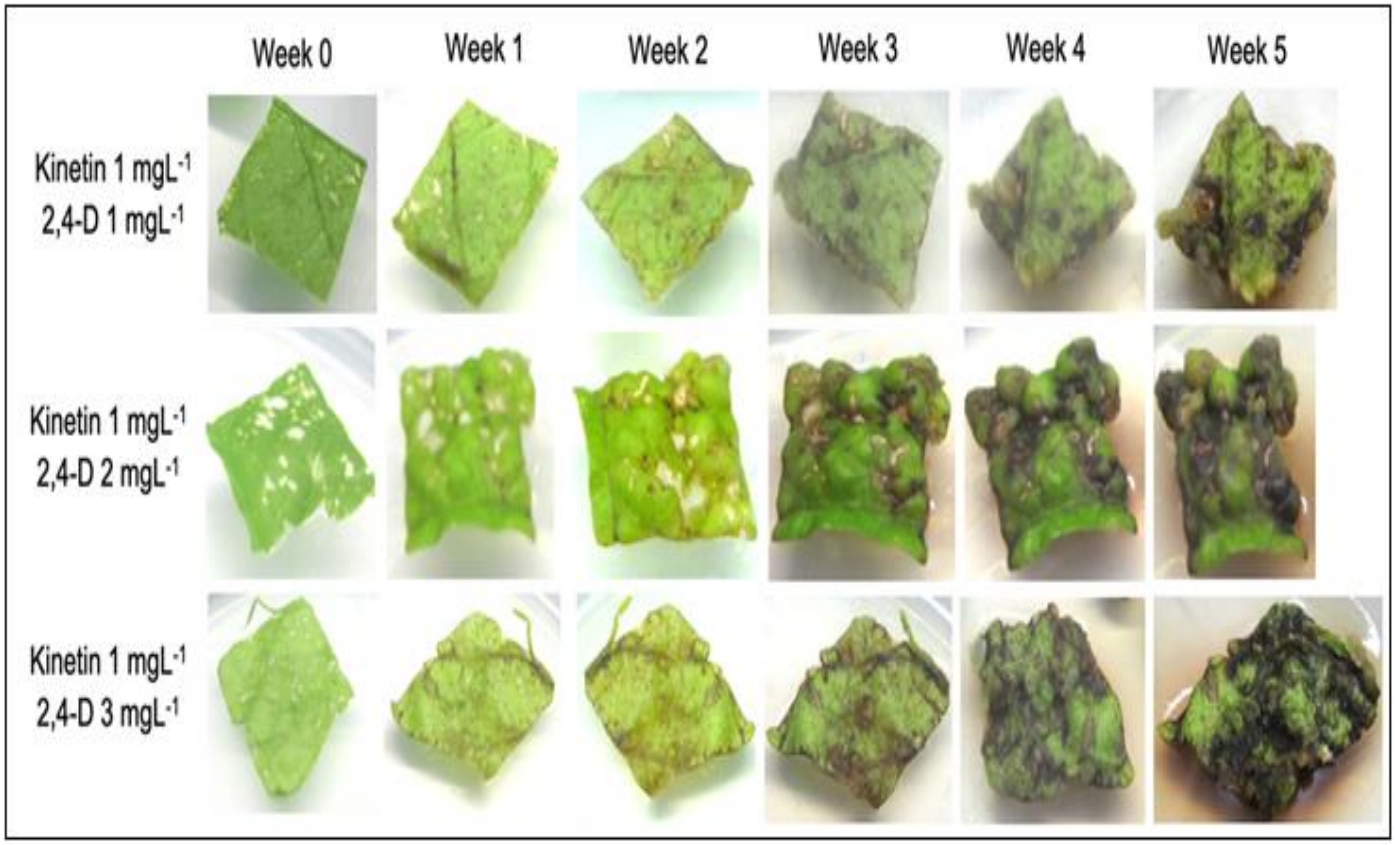

Fig 2 Effect of Combination of Kinetin and 2,4-D on the Indirect Somatic Embryogenesis from Leaf Explants of *C. ternatea*

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Regarding callus formation, Yucesan [2018] as in [34] demonstrated effectiveness at elevated TDZ concentrations. However, prolonged exposure and entirely excessive callus growth may lead to abnormal shoot growth, challenges in root formation, and necrosis. Hence, determining the critical concentration of TDZ is crucial for preserving the fate of tissue or cells in a plant tissue culture setup. TDZ, at concentrations exceeding 1 µM, has the potential to induce the formation of somatic embryos, adventitious shoots, and calluses [43,44,45]. In 26 lines of *Coffea arabica* plants, TDZ also influences the formation of embryogenic callus, with 2 mg^{L-1} TDZ identified as the concentration concentration demonstrating the greatest efficacy [46].

The use of TDZ in combination with other PGRs will affect the callus formation. In the in vitro regeneration of *Neolamarkia cadamba*, the combination of NAA and TDZ proved significantly more effective in triggering the development of adventitious buds compared to the combination of NAA and BA or NAA alone. Bud development took place via organogenesis facilitated by callus formation. In a study using leaf discs of *Arbutus andrachne* L., it was discovered that employing 0.5 mgL^{-1} NAA in combination with 3 mgL^{-1} TDZ yielded a callus induction rate of 66.66% within a span of 6 weeks. At the same NAA concentration, adding 1 mgL^{-1} TDZ and 2 mgL^{-1} TDZ showed a callus induction rate of 41.66% and 33.30%, respectively [47]. These results show that a concentration of 3 mgL^{-1} TDZ is the most effective concentration when combined with NAA to stimulate the rate of callus growth in leaf explants. Different types of explants can also cause differences in callus growth rate. The same study revealed that using 3 mgL^{-1} TDZ and 0.5 mgL^{-1} NAA on explant nodes could produce a callus growth rate of up to 100%. In this current study, the employed concentration of TDZ, which was 3 mgL^{-1}, proved to be the most effective concentration for inducing embryogenic callus originating from *C. ternatea* leaf explants. Wherein increased concentration of TDZ interrupted somatic embryo formation. This discovery reinforces a prior study that documented a decline in shoot organogenesis parameters as the TDZ concentration exceeded the optimal level [44].

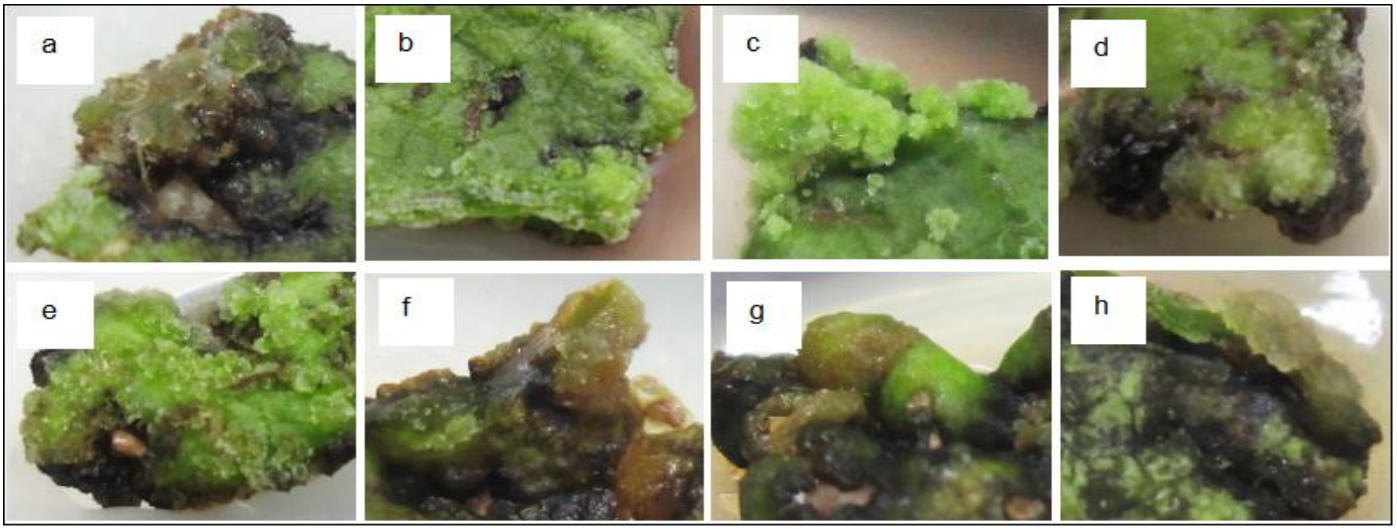

Fig 3 Effect of Different PGRs on the Indirect Somatic Embryogenesis from Leaf Explants of C. Ternatea after 5 Weeks of Cultured. a. MS + 1 mgL^{-1} TDZ; b. MS + 2 mgL^{-1} TDZ; c. MS + 3 mgL^{-1} TDZ; d. MS + 4 mgL^{-1} TDZ; e. MS + 5 mgL^{-1} TDZ; f. MS + 0.1 mgL^{-1} kinetin + 1 mgL^{-1} 2,4-D; g. MS + 0.1 mgL^{-1} kinetin + 2 mgL^{-1} 2,4-D; h. MS + 0.1 mgL^{-1} kinetin + 3 mgL^{-1} 2,4-D

In indirect somatic embryogenesis, the characteristics and consistency of embryogenic callus exhibited variability, potentially influenced by factors such as explant type, culture duration, and plant growth regulators (PGRs) [46]. It was observed that the use of different concentrations of PGRs in the media for callus formation from *C. ternatea* leaf explants caused a vary response in callus morphology. Induction with TDZ alone or in combination with kinetin and 2,4-D generated both non-embryogenic and embryogenic callus. These results are consistent with findings from earlier research conducted on immature embryos of maize [48]. They found that embryogenic callus, labeled as type II callus, displays a friable, less structured, almost granular surface. In contrast, non-embryogenic callus consists of soft, translucent tissue composed of cells incapable of plant regeneration. Additionally, it was noted that non-embryogenic callus had a somewhat brownish appearance compared to embryogenic callus. Embryogenic callus showed less oxidation than its non-embryogenic counterpart [49].

IV. CONCLUSION

Our findings indicate that the optimal choice for inducing a substantial amount of indirect somatic embryogenesis from *C. ternatea* leaf explants is 3 mgL^{-1} TDZ, serving as both the appropriate type and concentration of plant growth regulators (PGRs). This study introduces a fast and replicable regeneration technique that could prove beneficial for genetic transformation endeavors in *C. ternatea*.

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REFERENCES

[1]. N.K. Pandey, R.N. Tewari, G.C. Joshi, V.N. Pande, G. Padey, "Medicinal plants of Kumaon, Himalaya strategies for conservation," in Himalayan Biodiversity Conservation Strategies, U. Dhar, editor, Nainital, India: Himaviks Publications, 1993, pp. 293-302.

[2]. A.K. Bishoyi and K.A. Geetha, "Polymorphism in flower colour and petal type in Aparajita (*Clitoria ternatea*) ," *Open Access J Med Aromat Plants*, 2012, vol. 3, no. 2, pp. 12-14.

[3]. R. Mensah, D. Leach, A. Young, N. Watts and P. Glennie, "Development of *Clitoria ternatea* as biopesticide for cotton pest management: assessment of product effect on *Helicoverpa* spp. and their natural enemies," *Entomol. Exp Appl.*, 2015, vol. 154, no. 2, pp. 131–145.

[4]. G. Harada, S. Onoue, C. Inoue, S. Hanada and Y. Katakura, "Delphinidin-3-glucoside suppresses lipid accumulation in HepG2 cells," *Cytotechnology*, 2018, vol. 70, no. 6, pp. 1707–1712.

[5]. G.K. Oguis, E.K. Gilding, M.A. Jackson and D.J. Craik, "Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine," *Front Plant Sci*, 2019, vol. 10, pp. 645.

[6]. B.P. Devi, R. Boominathan and S.C. Mandal, "Anti-inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root," *Fitoterapia*, 2003, vol. 74, no. 4, pp. 345–349.

[7]. B. Parimaladevi, R. Boominathan and S.C. Mandal, "Evaluation of antipyretic potential of *Clitoria ternatea* L. extract in rats," *Phytomedicine*, 2004, vol. 11, no. 4, pp. 323–326.

[8]. N. Pengkumsri, K. Kaewdoo, W. Leeprechan and S.B. Sundaram, "Influence of extraction methods on total phenolic content and antioxidant properties of some of the commonly used plants in Thailand," *Pak J Biol Sci.*, 2019, vol. 22, no. 3, pp. 117-126.

[9]. S.A.T. Lakshan, C.K. Pathirana, N.Y. Jayanath and W.P.K.M. Abeysekara and W.K.S.M. Abeysekara, "Antioxidant and selected chemical properties of the flowers of three different varieties of butterfly pea (*Clitoria ternatea* L.) ," *Ceylon J Sci.*, 2020, vol. 49, no. 2, pp. 195-201.

[10]. N.K. Singh, D. Garabadu, P. Sharma, S.K. Shrivastava and P. Mishra, "Anti-allergy and anti-tussive activity of *Clitoria ternatea* L. in experimental animals," *J Ethnopharmacol.*, 2018, vol. 224, pp. 15–26.

[11]. I.U. Haq, G. Khurshid, A.Z. Abbasi, I. Nawaz, T. Naqvi, M. Arfan, *et al.*, "Successful callogenesis from leaf and petiole of *Bergenia ciliata* (Haw.) sternb and antibacterial activity of callus extracts," *Pak J Bot.*, 2019, vol. 51, no. 5, pp. 1847-52.

[12]. S.C. Teoh, S. Subramaniam and B.L. Chew, "The effects of 2,4-Dichlorophenoxyacetic acid on the induction of callus from cotyledon and hypocotyl explants of butterfly pea (*Clitoria ternatea*) ," *Malaysian Applied Biology*, 2023, vol. 52, no. 1, pp. 61-72.

[13]. G.K. Kumar and T.D. Thomas, "High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn," *Plant Cell Tiss Organ Cult.*, 2012, vol. 110, no. 1, pp. 141-151.

[14]. G.R. Rou, "Effect of cytokinins and auxins on micropropagation of *Clitoria ternatea* L," *Biol Lett.*, 2004, vol. 41, no. 1, pp. 21-26

[15]. C. Mohan and J.M. Upendra, "In vitro propagation of *Clitoria ternatea* L., a valuable medicinal plant," *Journal of Genetic Engineering and Biotechnology*, 2022, vol. 4, no. 2, pp. 217-220

[16]. F.A.O. Silveira, D. Negreiros, N.P.U. Barbosa, E. Buisson, F.F. Carmo, D.W. Cartensen, *et al.*, "ecology and evolution of plant diversity in the endangered *Campo Rupestre*: a neglected conservation priority," *Plant Soil*. 2016, vol. 403, pp. 129–152.

[17]. M. Ali, T. Isah and A. Mujib, "Climber plants: medicinal importance and conservation strategies," in Biotechnological strategies for the conservation of medicinal and ornamental climbers, A. Shahzad, S. Sharma, and S.A. Siddiqui, editors, Switzerland: Springer International Publishing, 2016, p. 101–138.

[18]. J. Wang, J-L. Li, J-X. Li, S-J. Lui, L.Q. Huang and W-Y. Gao, "Production of active compounds in medicinal plants: from plant tissue culture to biosynthesis," *Chin Herb Med.*, 2017, vol. 9, no. 2, pp. 115-125.

[19]. B. Sedaghati, R. Haddad and M. Bandehpour, "Efficient plant regeneration and agrobacterium-mediated transformation via somatic embryogenesis in Purslane (*Portulaca oleracea* L.): an important medicinal plant," *Plant Cell Tiss Organ Cult.*, 2018, vol. 136, no. 4, pp. 231-245.

[20]. U. Egertsdotter, I. Ahmad and D. Clapham, "Automation and scale up of somatic embryogenesis for commercial plant production, with emphasis on conifers," *Front Plant Sci.*, 2019, vol. 10, pp. 109.

[21]. P.P. Borpuzari and M. Borthakur, "Effect of plant growth regulators and explants sources on somatic embryogenesis of mature tissue of the anticancerous medicinal plant *Plumbago rosea*," *J Med Plants Stud*., 2016, vol. 4, no. 5, pp. 165-170.

[22]. W. Mose, A. Indrianto, A. Purwantoro and E. Semiarti, "The influence of Thidiazuron on direct somatic embryo formation from various types of explant in *Phalaenopsis amabilis* (L.) blume orchid," *HAYATI J Biosci*., 2017, vol. 24, no. 4, pp. 201-205.

[23]. H.A. Méndez-Hernández, M. Ledezma-Rodríguez, R.N. Avilez-Montalvo, Y.L. Juárez-Gómez, A. Skeete, J. Avilez-Montalvo, C. De-la-Peña and V.M. Loyola-Vargas, "Signaling overview of plant somatic embryogenesis," *Front Plant Sci*., 2019, vol. 10, pp. 77.

[24]. K. Pandeya, K.N. Tiwari, J. Singh, J.P. Verma and S.D. Dubey, "*In vitro* propagation of *Clitoria ternatea* l.: a rare medicinal plant," *J Med Plants Res.*, 2010, vol. 4, no. 8, pp. 664–668.

[25]. C.K. Roy, T.M. Mohiuddin, S.S. Rahman, "Establishment of *In vitro* micropropagation and callus formation protocols for large scale production of *Clitoria ternatea* L," *Z Arznei-Gewurzpfla*., 2017, vol. 22, pp.136-144.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/IJISRT24MAR149

[26]. A.M. Wójcik, B. Wójcikowska and M.D. Gaj, "Current perspective on the auxin-mediated genetic network that controls the induction of somatic embryogenesis in plants," *Int J Mol Sci*., 2020, vol. 21, no. 4, pp. 1333

[27]. L. Eyer, T. Vain, B. Pařizková, J. Oklestkova, E. Barbez, H. Kozubíková, "2,4-D and IAA amino acid conjugates show distinct metabolism in Arabidopsis," *PLoS ONE*, 2016, vol. 11, no. 7, pp. e0159269.

[28]. Y. Song, "Insight into the mode of action of 2,4-Dichloropehoxyacetic acid (2,4-D) as an herbicide," *J Integr Plant Biol*., 2014, vol. 56, no. 2, pp. 106-113.

[29]. X. Zhang, H. Li, Q. Qiu, Y. Qi, D. Huang and Y. Zhang, "2,4-Dichlorophenol induces global DNA hypermethylation through the increase of S-Adenosylmethionine and the upregulation of DNMTs mRNA in the liver of goldfish *Carassius auratus*," *Comp Bioschem Phys C*., 2014, vol. 160, pp. 54-59.

[30]. A. Fehér, T. Pasternak, Otvos, P. Miskolczi and D. Dudits, "Induction of embryogenic competence in somatic plant cells: a review," *Biol Sect Bot*., 2002, vol. 51, no. 1, pp. 5–12.

[31]. M.A. Zavattieri, A.M. Frederico, M. Lima, R. Sabino and B. Arnholdt-Schmitt, "Induction of somatic embryogenesis as an example of stress-related plant reactions," *Electron J Biotechnol*., 2010, vol. 13, no.1, pp. 12–13.

[32]. E.T. Dinani, M.R. Shukla, C.E. Turi, J.A. Sullivan and P.K. Saxena, "Thidiazuron: modulator of morphogenesis *in vitro*," in Thidiazuron: from urea derivative to plant growth, N. Ahmad and M. Faisal, editors, Singapore: Springer, 2018, p. 1-36.

[33]. J. Nisler, "TDZ: Mode of action, use and potential in agriculture," in *Thidiazuron: From urea derivative to plant growth*, N. Ahmad and M. Faisal, editors, Singapore: Springer, 2018, p. 37-59.

[34]. B. Yucesan, "Thidiazuron (TDZ): a callus minimizer for in vitro plant production" in Thidiazuron: from urea derivative to plant growth regulator, Singapore: Springer Narute, 2018, p.289-295.

[35]. M. Ebrahimi, A. Mokhtari and R. Amirian, "A highly efficient method for somatic embryogenesis of *Kelussia odorotissima* mozaff, an endangered medicinal plant," *Plant Cell Tiss Organ Cult*., 2018, vol. 132, no. 7, pp. 99-110.

[36]. N.A. Sherif, J.H.F. Benjamin, T.S. Kumar and M.V Rao, "Somatic embryogenesis, acclimatization and genetic homogeneity assessment of regenerated plantlets of *Anoectochilus Elatus* Lindl., an endangered terrestrial jewel orchid," *Plant Cell Tiss Organ Cult*., 2017, vol. 132, no. 2, pp. 303-316.

[37]. Z.D. Dalila, J. Hafsah, Z. Rokiah, K. Rodziah and M.N. Madihah, "Thidiazuron induces high frequency direct somatic embryogenesis growth from cotyledon culture of *Eurycoma longifolia*," *Sains Malays*., 2015, vol. 44, no. 7, pp. 913-920.

[38]. F-Y. Jheng, Y-Y. Do, Y-W. Liauh, J-P. Chung, P-L. Huang, "Enhancement of growth and regeneration efficiency from embryogenic callus cultures of *Oncidium* 'Gower Ramsey' by adjusting carbohydrate sources," *Plant Sci*., 2006, vol. 170, no. 6, pp. 1133-40.

[39]. B. Guo, B.H. Abbasi, A. Zeb, L. Xu and Y. Wei, "Thidiazuron: a multi-dimensional plant growth regulators: a mini review," *Afr J Biot*., 2011, vol. 10, no. 45, pp. 8984-9000

[40]. H. Huang, Y. Wei, Y. Zhai, K. Ouyang, X. Chen and L. Bai, "High frequency regeneration of plants via callus-mediated organogenesis from cotyledon and hypocotyl cultures in a multipurpose tropical tree (*Neolamarkia cadamba*)", *Scientific Reports*, 2020, vol. 10, pp. 4558.

[41]. S.C. Capelle, D.W. Mok, S.C. Kirchner and M.C. Mok, "Effects of Thidiazuron on cytokinin autonomy and the metabolism of N6-(Δ^2-isopentenyl) [8-14C] Adenosine in callus tissue of *Phaseolus lunatus* L," *Plant Phys*., 1983, vol. 73, no. 3, pp. 796-802.

[42]. T. Fujimura and A. Komamine, "Mode of action of 2,4-D and Zeatin on somatic embryogenesis in a carrot cell suspension culure," *Zeitsc für Pflanz*, 1980, vol. 99, pp. 1-8.

[43]. S. Mukhtar, N. Ahmad, M.I. Khan, M. Anis and M.A. Ibrahim, "Influencing micropropagation in *Clitoria ternatea* L. through the manipulation of TDZ levels and use of different explant types," *Physiol Mol Biol Plants*. 2012, vol. 18, no. 4, pp. 381-6.

[44]. M.A. Khan, B.H. Abbasi and A.K. Shinwari, "Thidiazuron enhanced regeneration and sylmarin content in *Silybum marianum* L," *Park J Bot*., 2014, vol. 46, no. 1, pp. 185-190.

[45]. A. Ghosh, A.U. Igamberiev and S.C. Debnath, "Thidiazuron-induced somatic embryogenesis changes of antioxidant properties in tissue culture of half-high blueberry plants," *Sci Rep*., 2018, vol. 8, no. 1, pp. 16978.

[46]. Y-C. Wang, M-Z. Lin, B. Huang, H-H. Chung, J-T Chen, "Thidiazuron enhanced somatic embryogenesis from callus lines of arabica coffee and subsequent plant regeneration," *Acta Biol Crac Ser Bot*., 2018, vol. 60, no. 2, pp. 35-44.

[47]. L.S. Sulaiman and R.S. Toma, "Callus initiation and plant organ regeneration of *Arbutus Andrachne* L. from leaves and nodes grown from *in vitro* seed culture," *Journal of University of Duhok*, 2023, vol. 26, no. 1, pp. 70-78.

[48]. M.E. Welter, D.S. Clayton, M.A. Miller and J.F. Petolino, "Morphotypes of friable embryogenic maize callus," *Plant Cell Rep*. 1995, vol. 14, no. 11, pp. 725-729.

[49]. V. Silveira, A.M. de Vita, A.M. Macedo, M.F.R. Dias, E.I.S Floh and C. Santa-Catarina, "Morphological and polyamine content changes in embryogenic and non-embryogenic callus of sugarcane," *Plant Cell Tiss Organ Cult*. 2013, vol. 114, pp. 351-364.